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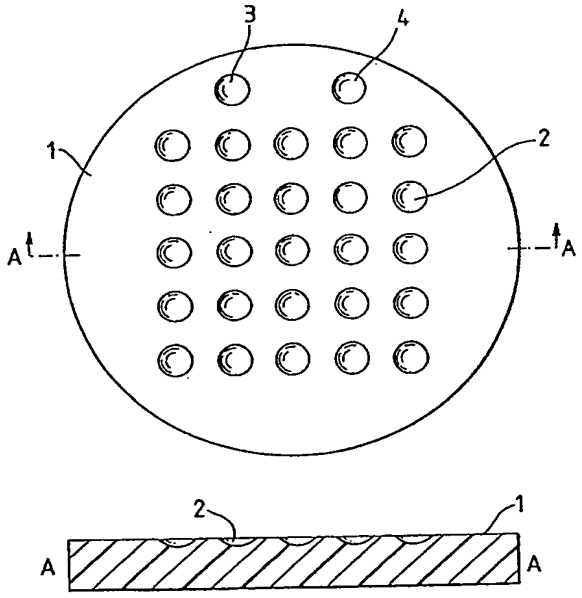
(54) Sample plate for immunosorbent electron microscopy

(57) A sample plate for immunosorbent electron microscopy is characterised in that the plate is made from polytetrafluoroethylene (PTFE). The plate preferably comprises a disc (1) of PTFE on one face of which a plurality of shallow wells (2) have been machined.



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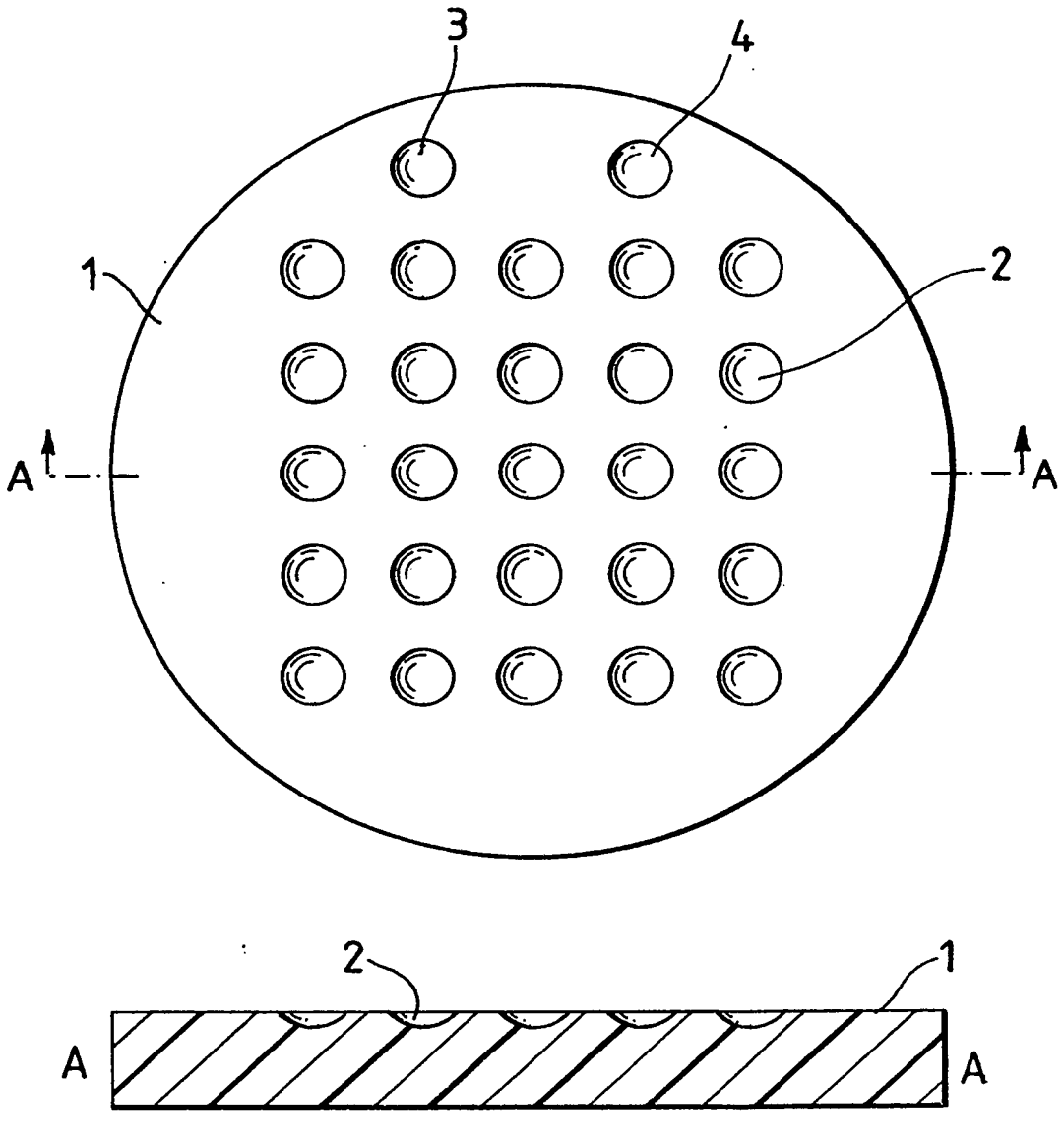
The drawing(s) originally filed was (were) informal and the print here reproduced is taken from a later filed formal copy.
The claims were filed later than the filing date within the period prescribed by Rule 25(1) of the Patents Rules 1982.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SPECIFICATION

Sample plate for immunosorbent electron microscopy

This invention relates to a sample plate for immunosorbent electron microscopy (ISEM) grid preparation. This is particularly but not exclusively useful for virus detection.

The detection and serological relationships of viruses may be readily effected by ISEM using the basic method originally described by Derrick, K.S. (1973); *Virology* 56, 652-653. Electron microscope grids coated with collodion and carbon are first floated on an antiserum preparation to allow antibody molecules to adsorb to their surfaces. The sensitised grids are then floated on droplets of test preparation to allow selective adsorption of homologous virus particles.

A wide variety of media have been used as supports for the serum and virus droplets. Common amongst these are glass plates and dishes (native or siliconized), polystyrene plates and Petri dishes and paraffin wax film. There are several drawbacks associated with each of these materials.

a. Antibody coated grids are commonly floated on test sample droplets for 2-18 hours. Vibration causes droplet spreading and increased rate of evaporation and possible deposition of the grid on to the support often with damage to the coating.

b. Threat of droplet spreading means that large space allowances must be made between droplets to prevent cross contamination.

c. The use of detergents in preparation of test samples vigorously promotes droplet spreading.

d. There may be significant non-specific adsorption of antibody and sample by uncoated glass and particularly polystyrene (see Allen, R.T., Buckland, R.A. and Tyrrell, D.A.J. (1973); *Arch Gesamte Virusforsch.* 41, 394-397).

The present invention provides a sample plate for immunosorbent electron microscopy, characterised in that the plate is made from polytetrafluoroethylene (PTFE).

Sample plates made from PTFE in which shallow wells have been machined have proved to be successful for immunosorbent electron microscopy (ISEM) grid preparation. The plates are easily made, precisely locate each sample even when detergent is present, exhibit no non-specific sample adsorption effects and are readily cleaned and sterilized for reuse.

Reference is now made to the accompanying drawing, which is a plan view and vertical section on line A-A of a PTFE sample plate according to a preferred embodiment of the invention.

While PTFE rod of 60 mm nominal diameter was purchased from Amari Plastics Ltd., Chickenhall Lane, Eastleigh, Hants. SO6 5RR. As a reasonable compromise between cost, anticipated sample numbers, handling ease and rigidity for machining, the rod was cut into discs 1 approximately 7.5 mm thick. A pattern of wells 2 was then cut in each as shown in the drawing using a 3/16 in. (4.76 mm) bull-nosed slotting drill. Wells were cut with a centre-to-centre spacing of 8 mm and to a depth of 1.5 mm. (The latter was decided by trial and error based on a grid diameter of 3 mm and a desire to economise on antiserum by using droplets of 10-15 μl.) The resulting depressions showed clean edges and smooth surfaces and no further treatment was necessary.

When in use sample plates were kept in a humid chamber, usually a 9 cm plastic Petri dish containing a disc of damp filter paper. The latter served also to prevent lateral movement of the plate due to the low coefficient of friction of PTFE. A droplet size of 10-12 μl was found to be quite adequate although 12 μl was used when detergent was present.

The shape of the wells was found to be beneficial in the few cases where grids sank through the droplets. As grids are supported by the edges only on the spherical surface there is a much greater likelihood of recovery intact compared with the damage normally done peeling grids from a flat surface when firmly held by surface tension of a well spread droplet.

Sample wells were cut in a regular pattern to assist row-by-row processing, but two additional wells 3,4 were cut to serve for plate orientation and for reference or control samples. Twenty five wells per plate was felt to be a reasonable number to process as one batch but larger diameter PTFE rod is available and many more wells can be fitted on to such discs.

The great chemical resistance of PTFE allows use of samples prepared in, or processed using, organic solvents. It also allows cleaning after use by boiling in strong detergents and sterilization in hot air ovens - particularly helpful when working with highly infectious viruses. After 18 months of continuous treatment, plates remain in excellent condition.

Of great importance is the freedom from non-specific adsorption effects of PTFE. To examine this, various support media were tested for protein adsorption by a simple ELISA* test. Samples of test materials were exposed to 10 μl droplets of purified rabbit antibody diluted to 1.0 and 0.1 μg protein/ml. After 30 min test pieces were washed free of unbound protein and guinea pig-anti rabbit-alkaline phosphatase conjugate applied for 60 min. After washing, substrate was applied for 30 min. The reaction was then stopped and the colour density was determined. The means of several tests are shown in Table 1.

* ELISA = enzyme-linked immunosorbent assay, described for example by Clark M.F. and Adams A.N., J.gen.Virol. (1977), *34*, 475-483.

Table 1
Non-specific adsorption effects of ISEM support media as measured by ELISA

Support medium	*E405 at sample concn.*	
	1 μg/ml	*0.1 μg/ml*
Glass (native)	0.167	0.086
Glass (siliconized)	0.020	0.000
Polystyrene tube	0.240	0.119
ELISA plate	0.384	0.272
PTFE	0.000	0.000
Parafilm	0.026	0.003

As expected, polystyrene ELISA plates had strong adsorption characteristics and polystyrene tubes behaved in a similar manner (see Allen *et al* referred to above). Untreated glass showed moderate non-specific adsorption whereas siliconized glass and parafilm were very significantly better. No protein adsorption occurred with PTFE. Although these last three named materials are suitable inert supports for ISEM only PTFE allows easy shaping to provide sample wells.

Sample plates made of PTFE are easily machined to provide sample retaining wells for ISEM. Their lack of non-specific adsorption effects, resistance to chemical attack and sturdiness for reuse makes them a most useful low cost accessory for ISEM sample preparation.

CLAIMS

1. A sample plate for immunosorbent electron microscopy, characterised in that the plate is made from polytetrafluoroethylene (PTFE).

2. A sample plate according to claim 1, comprising a disc of PTFE on one face of which a plurality of shallow wells have been machined.

3. A sample plate according to claim 2, in which the wells are regularly arranged in a plurality of rows on the face of the disc.

4. A sample plate for immunosorbent electron microscopy, substantially as hereinbefore described with reference to and as shown in the accompanying drawing.

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